Title: Evaluating Neuroprotective Effects of The Combination of Fingolimod and Alteplase in Acute Ischemic Stroke: A Randomized Clinical Trial

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To appear in: **Basic and Clinical Neuroscience**

Received date: 2025/12/22

Revised date: 2026/03/22

Accepted date: 2026/04/18

Please cite this article as:

Shahriyari, H., Abdi, S., Mohebbi, N., Fatehi, F., Dirandeh, E., Soleimani, S., et al. (In Press). Evaluating Neuroprotective Effects of The Combination of Fingolimod and Alteplase in Acute Ischemic Stroke: A Randomized Clinical Trial. Basic and Clinical Neuroscience. Just Accepted publication Jul. 10, 2026. Doi: http://dx.doi.org/10.32598/bcn.2026.8493.1

DOI: http://dx.doi.org/10.32598/bcn.2026.8493.1

Abstract

Objectives and aims: Ischemic stroke, as one of the most common neurological conditions, has debilitating consequences both in mortality and morbidity for which there is only revascularization as a highly effective treatment; considering the mentioned fact, a great and varied effort has been shaped during the last decades to add neuroprotective agents for a better outcome; however, there are both knowledge and practical gaps. In this study, we aimed to evaluate the impact of Fingolimod as a neuroprotective agent by reducing necroptosis through immunomodulation on clinical outcomes in stroke patients, as an adjunct to standard care.

Methods and Materials: This clinical trial was conducted in 2021 and 2024 at Tehran Shariati Hospital. The study protocol received approval from the Institutional Review Board (IRB) of Tehran University of Medical Sciences under the ethical approval code **IR.TUMS.MEDICINE.REC.1398.5.99** and registered in the Iranian registry of clinical trials **(IRCT20220423054619N1)**. The study sample included patients with ischemic stroke who presented to the ER within 4.5 hours after the onset of focal neurologic deficit and NIHSS of 6 or higher, which, in brain CT scan, there is no better explanation. We excluded strictly any patient with any block or old or new MI, due to Fingolimod complications. Patients were randomly assigned in a block of four to two classic and Fingolimod groups. In the first group, patients only received Alteplase (0.9 mg/kg). Based on the consensus of the literature, in the second group, Fingolimod was administered alongside Alteplase for three consecutive days (0.5 mg orally) which was adapted from the most common protocol used in previous studies, with particular reference to the study of zhu et al. mRS and NIHSS questionnaires were used before injection, 30 days, and 90 days after treatment. mRS and NIHSS scores were considered as primary outcomes of the study. Leukocyte major subtypes (CD4 and CD8) were also gathered in each group on day 1 and day 3. Data were analyzed with SPSS version 22. The incidence of strokes amenable to intervention and Fingolimod complications was analyzed between the two groups, with no significant difference. So, it is safe if a proper criterion is used.

Results: A total of 27 patients in the classic and 25 patients in the Fingolimod groups were evaluated. Baseline demographic. The mRS was initially comparable, but by days 30 and 90, a statistically significant difference emerged between the groups (p = 0.041 on day 30 and p = 0.030 on day 90). NIHSS was significantly improved in the Fingolimod group at day 90 post-stroke. (p= 0.026). A trend toward reduction was detected for leukocyte subtypes in the Fingolimod group on day 3, with a non-significant 26% reduction in CD4+ cells (p = 0.072) and a statistically significant 31% reduction in CD8+ cells (p = 0.008).

Conclusion: Fingolimod plus Alteplase may be associated with improved neurologic function in ischemic stroke patients compared to Alteplase alone. The combination was well-tolerated, with a safety profile comparable to Alteplase monotherapy. Further research is needed before clinical implementation can be recommended**.**

Keywords: Fingolimod, Stroke, Alteplase, Neuroprotective agents

Introduction

Ischemic stroke is defined as infarction of brain tissue due to the cessation of blood flow, which rapidly develops brain dysfunction, is a serious cause of morbidity and mortality. In fact, in 2019, it was the second-leading cause of death and disability combined, accounting for 5.7% of total DALYs worldwide (Feigin et al., 2021). Despite a declining trend in age-standardized rates, the overall burden is increasing due to aging populations and persistent risk factors (Zhu et al., 2024) Currently, Alteplase as a recombinant tissue-type plasminogen activator (rTPA), is the only available FDA-approved therapeutic choice for patients with ischemic stroke who have experienced symptoms within 4.5 hours(Hacke et al., 2008)Although Alteplase is an approved agent in these patients, it is associated with several complications, even in patients who fully meet the indications of Alteplase administration(Jaillard et al., 1999; "Tissue Plasminogen Activator for Acute Ischemic Stroke," 1995). Alteplase acts via reperfusion in ischemic areas of the brain and associated reperfusion injury is inevitable in some cases. Hemorrhagic transformation and massive edema are among the most common reperfusion injuries consequent to the administration of Alteplase(Balami et al., 2013; Zhu et al., 2015). Spontaneous hemorrhagic transformation has been reported with an incidence of 38-71% in autopsy and 1343% in clinical trial studies. (Bang et al., 2011; Jaillard et al., 1999) The risk of intracranial hemorrhage has been reported to be 10-fold higher with Alteplase treatment compared to untreated patients("Tissue Plasminogen Activator for Acute Ischemic Stroke," 1995). Hemorrhagic transformation occurs early in the course, with 80% of cases happening within 12 hours after receiving rTPA(Group, 1997). Brain edema also initiates several hours after the onset of stroke, increases up to day 8, and then gradually disappears up to day 30(Bang et al., 2011; Schwamm et al., 1998; Simard et al., 2007). The compressive effect of severe edema is a prominent cause of death in ischemic stroke patients(Simard et al., 2007)

On the one hand, although rTPA has a significant effect, its efficacy remains unsatisfactory; on the other hand, given the issues, concerns have been raised about the administration of Alteplase in patients with various conditions, making researchers attempt to find newer agents for ischemic stroke. The neuroprotective options can be classified into two categories: nonpharmacological, such as remote ischemic conditioning, and pharmacological. The pharmacological options are mainly focused on two pathophysiologic mechanisms; firstly, they can be affected by reducing oxidative stress, like edaravone, cerebrolysin, citicoline, etc., or by reducing inflammation, which is the main goal of our study (Bornstein et al., 2018; Dávalos et al., 2012; Zhao et al., 2024). Immune-inflammatory mechanisms possess major roles in the development of ischemic stroke and its consequent reperfusion injuries(Ahmad & Graham, 2010; Liantao et al., 2019; Wang et al., 2025). To address these inflammatory mechanisms in stroke and post-stroke settings, a huge number (>1000) of neuroprotective agents have been tested without any significant clinical benefit (O'Collins et al., 2006) , but in recent years, Fingolimod, a sphingosine analog, has shown signals of promising results in a few studies of ischemic stroke patients. Fingolimod, which was first introduced as an oral agent for relapsing-remitting forms of multiple sclerosis, acts through the sphingosine-1-phosphate receptor(K. Schuhmann et al., 2016; Kraft et al., 2013; Tian et al., 2018; Zhang et al., 2025). The cascade of actions following its binding to the S1P receptors on lymphocytes inhibits the engagement of lymphocytes in the inflammatory process. This inhibition of inflammatory mechanisms yields a state of immunosuppression in the central nervous system(Attari et al., 2016; Enriquez-Marulanda et al., 2017). It can be assumed that inhibition of inflammation can aid prevention and attenuation of post-thrombolysis reperfusion injuries. (Liantao et al., 2019) Several studies have suggested that Fingolimod can diminish the growth of cerebral edema and infarction, maintain blood-brain barrier permeability, and significantly improve outcomes of patients’ neurological functions (Bai et al., 2022; Hou et al., 2015; Liantao et al., 2019).to expand on this, a network metanalysis which assessed 5 human clinical trials showed safety and efficacy using a random-effects model;(Bai et al., 2022) of note, one study which was in Chinese did not show superiority of the Fingolimod above standard care. (Bai et al., 2022; 田德财, 2017) This agent is also involved in the inhibition of platelet-activating factor receptors and platelet aggregation which can be theoretically beneficial in the reduction of occlusion of arteries and cardiovascular risk(Attari et al., 2016) A well designed pilot study by Zhu et al. suggests that inflammatory and immune responses triggered by cerebral ischemia can worsen clinical outcomes of stroke by contributing to hemorrhagic transformation, massive edema, and reperfusion injury when treated with intravenous Alteplase. This study investigated the safety and efficacy of combining Fingolimod and Alteplase to mitigate reperfusion injury in patients with acute ischemic stroke within the first 4 hours of symptom onset. The results were promising both in efficacy and safety, and its usage of flow cytometry on days 1, 3, and 7 highlighted the probable inflammation-based interference of the medication. (Zhu et al., 2015) Of note, most of the studies were performed in East Asia(Aljabali et al., 2023; Zhao et al., 2024). The most cited studies were just evaluator-blinded clinical trials, and the participants remained at a minimum, in which all of them reported promising results with different effect sizes, and there was no significant complication (Aljabali et al., 2023; Liantao et al., 2019; Tian et al., 2018; Zhao et al., 2024; Zhu et al., 2015) of note, new generation of sphingosin-1-receptor antagonists has less cardiac effect mitigating potential cardiac worrisome in bedside and opens our hand to make progress in this field more flexible. (Long et al., 2021) searching through clinical trials, there are many ongoing trials that are being pursued in combination with alteplase and a shift to alteplase and mechanical thrombectomy, showing the importance of our study.

At present, Data about Fingolimod usage in acute ischemic stroke is still limited, particularly regarding the extent of the effect, and clinical safety; besides, the studied population was at a minimum, limited to a geographical and ethnical group, whereby further studies for elucidation of its role in ischemic stroke are needed.

This study offers a novel contribution by evaluating the clinical efficacy of combining Fingolimod with Alteplase, compared to standard Alteplase monotherapy, in the treatment of acute ischemic stroke. Although the immunomodulatory potential of Fingolimod has been previously proposed in experimental contexts, its integration into acute stroke management remains underexplored in clinical settings. Our investigation advances this field by implementing a rigorous methodological framework, including stringent and inclusive case selection criteria, a triple-blind design to minimize bias, and the assessment of a distinct patient population. These methodological enhancements not only improve internal validity but also facilitate patient adherence and protocol feasibility. By addressing existing gaps in translational application, this study establishes a critical foundation for future multi-center clinical trials aimed at evaluating the appropriateness of incorporating Fingolimod into standard stroke care.

Material and Methods

This clinical trial was conducted in 2021-2024 at Shariati Hospital, Tehran, Iran. The study protocol received approval from the Institutional Review Board (IRB) of Tehran University of Medical Sciences under the ethical approval code **IR.TUMS.MEDICINE.REC.1398.5.99** and registered in the Iranian registry of clinical trials **(IRCT20220423054619N1)**. This triple-blind randomized controlled trial ensured that patients, treating physicians, and outcome assessors were all blinded to treatment allocation. Medication preparation was performed by an independent pharmacist not involved in patient care or assessment. The study population consisted of patients with the onset of ischemic stroke within the previous 4.5 hours who had clinical symptoms compatible with a diagnosis of stroke, having at least an NIHSS≥6, which were confirmed with a CT scan. Informed consent was taken from patients or their families. A complete history was taken, and a physical examination was performed. Patients with long QT, sensitivity to drugs, consumption of anti-arrhythmic agents, second and third-degree cardiac block, heart failure, history of myocardial infarction, transient stroke, and unstable angina within the previous 6 months were excluded from the study. Sample size was calculated using G*Power (version 3.1) with α = 0.05 and a desired power of 80%. However, final enrollment did not reach this target due to inclusion/exclusion criteria, particularly the exclusion of patients with cardiac conduction abnormalities given Fingolimod’s known bradycardic effects, as well as the limited time window for thrombolysis eligibility (4.5 hours) and the challenges of patient recruitment during the COVID-19 pandemic period (2021–2024). Given these limitations, the final sample size was 52 participants, consistent with the exploratory nature of this clinical trial. Patients were randomly assigned to two groups. Considering the small number of participants, we chose to assign them in a block of four randomization. In the first group, classic treatment with Alteplase (0.9 mg/kg) was performed if the patient fulfilled the criteria. In the second group, in addition to routine treatment with Alteplase, Fingolimod was used as well, using the protocol that follows. Fingolimod was administered for three consecutive days (0.5 mg daily via oral route). Treatment was initiated within 4.5 hours after the onset of ischemic stroke symptoms. Modified Rankin Scale (mRS) and National Institute of Health Stroke Scale (NIHSS) questionnaires were used at baseline, one month later, and three months later. mRS and NIHSS scores were considered as study outcomes. Scoring was done by an individual blinded to the study and unaware of the treatment received by the patient. mRS is a widely used scale for measuring disability or dependence level of patients after a stroke. The scale scoring is as follows: 0 (absence of symptoms), 1 (no remarkable disability, able to perform all daily activity despite presence of some symptoms), 2 (minor disability, no need to help but unable to perform all previous activities), 3 (moderate disability, needs help in daily activities but can walk alone), 4 (moderate-severe disability, needs help in daily activities and walking), 5 (severe disability, bedridden, needs help for any activity), 6 (dead) (Banks & Marotta, 2007). NIHSS score is also a 15-item scale for the assessment of neurologic outcomes and recovery status of stroke patients (Table 1) (Kwah & Diong, 2014). Apart from neurological function, the leukocyte subtypes of CD4 and CD8 were counted by flow cytometry in our center on day 1 and day 3, thereby assessing the Fingolimod mechanism of action correlation with clinical data. We also compared the past medical history and assessed the location of arterial stenosis by MR angiography after treating the emergent condition and finding a stable condition. Major post-stroke complications based on the literature were also assessed at the end of 3 months.

1a—Level of consciousness	0 = Alert; keenly responsive 1 = Not alert, but arousable by minor stimulation 2 = Not alert; requires repeated stimulation 3 = Unresponsive or responds only with reflex
1b—Level of consciousness questions: What is your age? What is the month?	0 = Answers two questions correctly 1 = Answers one question correctly 2 = Answers neither questions correctly
1c—Level of consciousness commands: Open and close your eyes Grip and release your hand	0 = Performs both tasks correctly 1 = Performs one task correctly 2 = Performs neither task correctly
2—Best gaze	0 = Normal 1 = Partial gaze palsy 2 = Forced deviation
3—Visual	0 = No visual lost 1 = Partial hemianopia 2 = Complete hemianopia 3 = Bilateral hemianopia
4—Facial palsy	0 = Normal symmetric movements 1 = Minor paralysis 2 = Partial paralysis 3 = Complete paralysis of one or both sides
5—Motor arm Left arm Right arm	0 = No drift 1 = Drift 2 = Some effort against gravity 3 = No effort against gravity 4 = No movement
6—Motor leg Left leg Right leg	0 = No drift 1 = Drift 2 = Some effort against gravity 3 = No effort against gravity 4 = No movement
7—Limb ataxia	0 = Absent 1 = Present in one limb 2 = Present in two limbs
8—Sensory	0 = Normal; no sensory loss 1 = Mild-to-moderate sensory loss 2 = Severe-to-total sensory loss
9—Best language	0 = No aphasia; normal 1 = Mild-to-moderate aphasia 2 = Severe aphasia 3 = Mute; global aphasia
10—Dysarthria	0 = Normal 1 = Mild-to-moderate dysarthria 2 = Severe dysarthria
11—Extinction and inattention	0 = No abnormality 1 = Visual, tactile, auditory, spatial, or personal inattention 2 = Profound hemi-inattention or extinction
Score = 0–42	

Table 1 National Institute of Health Stroke Scale (NIHSS) scoring system

Patients who were not willing to continue with the study were excluded. SPSS for Windows version 22.0 software (IBM, Armonk, NY) was used for statistical analysis. For descriptive analysis, mean and percentage as central indices and standard deviation as variance indices were used. Categorical parameters were reported as frequency and percentage. The t-test was used for age due to its parametric character to compare quantitative parameters, and the Mann-Whitney U test was for NIHSS and mRS score for its nonparametric distributions. It is worth noting that the results were compared both across groups at each time point and within each group over time. The chi-square test was used for the comparison of categorical parameters. Statistical significance was considered as p-value <0.05. Effect sizes were calculated using non-parametric rank-based methods appropriate for ordinal outcomes. Odds ratios were derived from dichotomized outcomes with 95% confidence intervals, in which all comparisons were performed against baseline characteristics. The ethics committee of the Tehran University of Medical Sciences approved the study.

Results

56 eligible patients were reviewed and enrolled in our study. During the study, four participants were excluded due to the loss of connection for evaluation all of them were before the first (30-day) follow-up. Finally, 52 patients completed the study with 27 patients in the classic group (Alteplase alone) and 25 patients in the Fingolimod group. There were five deaths during the study: three in the Classic group and two in the Fingolimod group. Two deaths occurred before the first (30-day) evaluation and three after that time.

The mean age in the classic and Fingolimod groups was 69.3 ± 14.7 and 68.8 ± 9.1 years, respectively (p = 0.621). In the classic group, 19 patients (70.37%) were male and 8 (29.62%) were female, while in the Fingolimod group, 16 patients (64%) were male and 9 (36%) were female (p = 0.344). Underlying conditions were evaluated and are summarized in Table 2. These analyses did not reveal statistically significant differences in baseline demographic characteristics or comorbidities, suggesting that the two groups were reasonably well-matched.

Table 2 Underlying conditions in patients of the classic and Fingolimod groups

Underlying condition	Classic group	Fingolimod group	P-value
Hypertension	15 (55%)	13 (52%)	0.811
Diabetes mellitus	6 (22.22%)	7 (28%)	0.297
Hyperlipidemia	12 (44.44%)	11 (40.74%)	0.505
Atrial fibrillation	3 (11.11%)	2 (8%)	0.119
Smoking	14 (51.85%)	13 (52%)	0.838
History of stroke	4 (14.81%)	5 (20%)	0.272

Due to the potential influence of antiplatelet and anticoagulant medications, these baseline data are presented and analyzed as follows: antiplatelet agents were used by 7 patients (25.92%) in the classic group and 5 patients (20%) in the Fingolimod group (p = 0.329). Anticoagulants were administered to 1 patient (3.7%) in the classic group and 1 patient (4%) in the Fingolimod group (p = 0.944). Another potential confounding factor, onset-to-treatment time, was also evaluated; the mean onset-to-treatment time was 3.2 ± 0.4 hours in the classic group and 3.1 ± 0.3 hours in the Fingolimod group (p = 0.733). Based on MR angiography findings, most arterial occlusions were located in the second segment of the middle cerebral artery. Details of the occlusion sites are provided in Table 3.

Table 3 Locations of arterial occlusion in classic and Fingolimod groups

Artery	Classic group	Fingolimod group	P-value
Anterior cerebral artery	1 (3.7%)	0 (0%)	0.280
Proximal section of the first segment of middle cerebral artery	1 (3.7%)	2 (8%)	
The middle section of the first segment of the middle cerebral artery	2 (7.40%)	1 (4%)	
The distal section of the first segment of the middle cerebral artery	3 (11.11%)	4 (16%)	
The second segment of the middle cerebral artery	17 (62.96%)	15 (60%)	
Terminal internal carotid artery	3 (11.11%)	3 (12%)	

At baseline, there were no significant differences between the groups in mRS scores ($p > 0.05$), confirming that the groups were well-matched initially. At 30-day follow-up, the Fingolimod group showed superior functional recovery (median mRS 2 [IQR 1-3]) compared to the Classic group (3 [3-4]; p=0.041). A greater proportion of Fingolimod-treated patients achieved good functional outcomes (mRS 0-2: 52% vs 14.8%; OR 6.21, 95% CI 1.7-22.6). By 90 days, the between-group difference in functional outcomes increased further. The Fingolimod group maintained a median mRS of 2 [0-2] versus 3 [2-4] in the Classic group (p=0.030). The proportion of patients with mRS of 2 was 68% in the Fingolimod group compared to 22.2% in the Classic group. The results of mRS are shown in Table 4.

Table 4 Evaluation of mRS in three time periods (Classic vs, Fingolimod)

mRS	Classic group	Fingolimod group	P-value
Baseline Median (IQR)	4 (3-4)	4 (3-4)	0.757
0	0 (0%)	0 (0%)	
1	1 (3.7%)	1 (4%)	
2	0 (0%)	1 (4%)	
3	16 (59.25%)	12 (48%)	
4	7 (25.92%)	8 (32%)	
5	1 (3.7%)	1 (4%)	
6	2 (7.40%)	2 (8%)	
30-day mRS Median (IQR)	3 (3-4)	2 (1-3)	0.041
0	1 (3.7%)	2 (8%)	
1	1 (3.7%)	2 (8%)	
2	2 (7.40%)	9 (36%)	
3	15 (55.55%)	6 (24%)	
4	6 (22.22%)	3 (12%)	
5	0 (0%)	2 (8%)	

6	2 (7.40%)	1 (4%)	
90-day mRS	3 (2-4)	2 (0-2)	0.030
0	1 (3.7%)	3 (12%)	
1	2 (7.40%)	3 (12%)	
2	3 (11.11%)	11 (44%)	
3	13 (48.14%)	4 (16%)	
4	5 (18.51%)	2 (8%)	
5	2 (7.40%)	1 (4%)	
6	1 (3.70%)	1 (4%)	

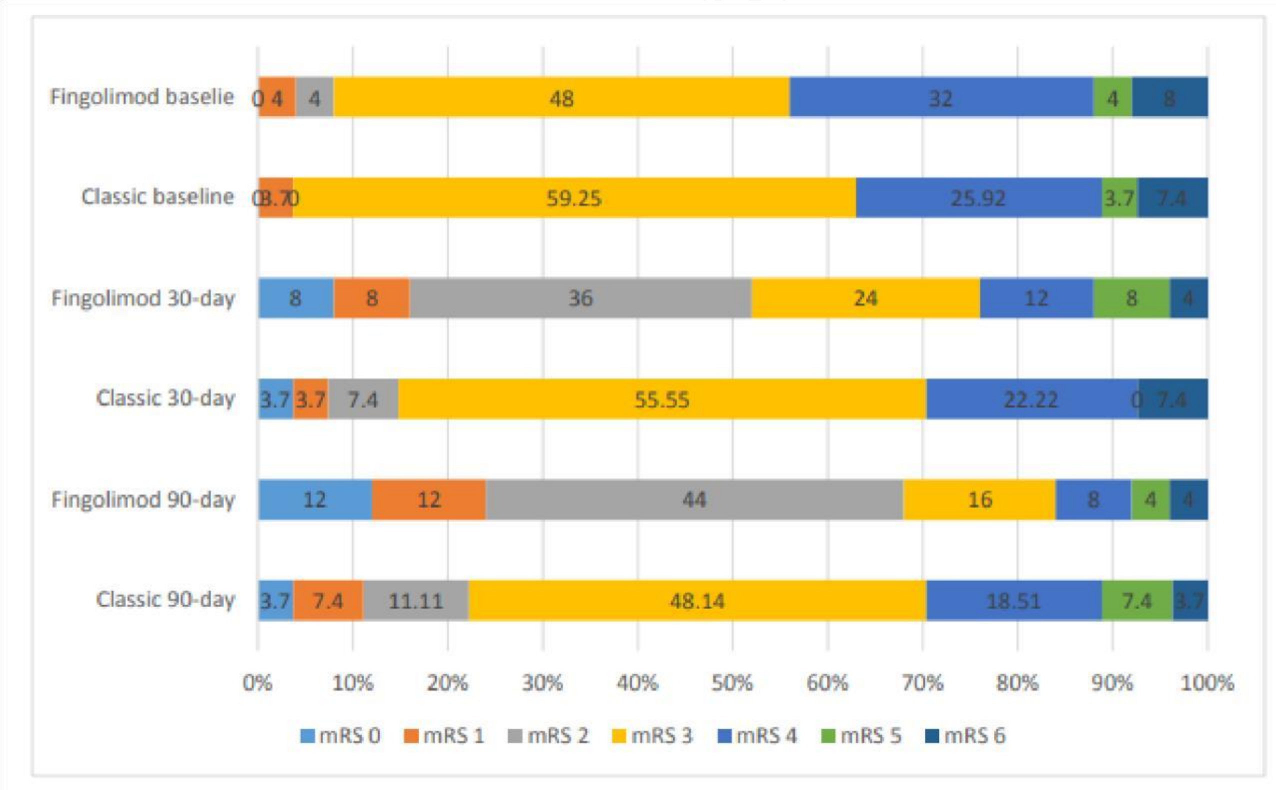

Figure 1 distribution of mRS scores in classic and Fingolimod groups during the study

At baseline, both groups had similar disability profiles, with most patients scoring mRS 3-4 (Figure 1). By 90 days, the Fingolimod group showed a marked shift toward functional independence: 68% achieved mRS 0-2 compared to 22% in the Classic group (p=0.030). Specifically, 12% of Fingolimod patients achieved complete recovery (mRS 0) versus 3.7% in the Classic group, and 44% achieved mRS 2 (slight disability) versus 11.11% in the Classic group. The odds of achieving functional independence (mRS 0-2) at 90 days were significantly higher in the Fingolimod group (OR 6.21, 95% CI 1.7–22.6; p=0.030).

Baseline NIHSS scores (Table 5) were similar between groups (Classic: 12 [4-20] vs Fingolimod: 14 [7-25]; p=0.135). At 30 days, both groups showed improvement (Classic: 5 [2-18] vs Fingolimod: 3 [1-16]; p=0.059). By 90 days, the Fingolimod group demonstrated significantly lower NIHSS scores (Classic: 3 [1-18] vs Fingolimod: 1 [0-12]; p=0.026) (Figure 2).

Table 5 Evaluation of NIHSS in three time periods (Classic vs, Fingolimod)

NIHSS	Classic group Median (IQR)	Fingolimod group Median (IQR)	P-value
Baseline	12 (4-20)	14 (7-25)	0.135
30-day	5 (2-18)	3 (1-16)	0.059
90-day	3 (1-18)	1 (0-12)	0.026

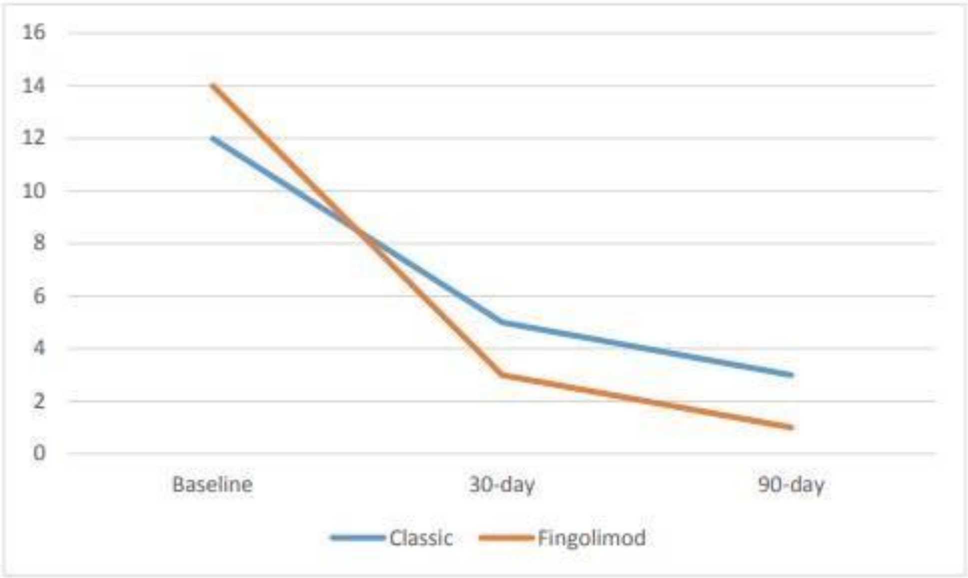

Figure 2 Comparison of NIHSS score in the course of study (Classic vs, Fingolimod)

The effect sizes were analyzed as follows: The rank-biserial correlation increased from 0.33 (30-day) to 0.38 (90-day) for mRS scores, with corresponding odds ratios of 6.21 (95% CI 1.7-22.6) and 7.44 (95% CI 2.3-24.1) for achieving mRS 0-2. NIHSS improvements demonstrated smaller but significant effects (30-day r=0.21; 90-day r=0.29), with a 90-day OR of 3.19 (95% CI 1.1-9.3) for NIHSS ≤1. The number needed to treat improved from 3 to 2 for mRS and from 5 to 4 for NIHSS between 30- and 90-day assessments.

In longitudinal analysis, Patients beginning Fingolimod plus alteplase treatment started with significant disability (median mRS 4) but showed remarkable progress within just one month. The early improvements were both statistically significant (p<0.001) and clinically meaningful, with median disability scores dropping to mild-moderate levels (mRS 2). This positive trajectory continued through the 90-day mark, where further subtle but significant gains were observed (p=0.043). In terms of NIHSS, these patients demonstrated particularly robust early recovery, cutting their NIHSS scores by nearly 80% in the first month (p<0.001), with continued gradual improvement thereafter (p=0.012).

Patients receiving standard care also made important strides in their recovery, though the pace and extent differed. From equally impaired starting points, this group achieved moderate but statistically significant functional improvement by 30 days (p=0.002), though subsequent gains were more modest and did not reach statistical significance (p=0.118). NIHS score improvements followed a steadier pattern, with significant reductions at each timepoint (30-day p<0.001; continued 90-day improvement p=0.009).

No significant difference in terms of complications was found between the two groups. Adverse events in the course of study are listed in Table 6.

Table 6 Post-stroke complications

Complication	Classic group	Fingolimod group	P-value
Mortality	3 (11.11%)	2 (8%)	0.271
Myocardial infarction	1 (3.70%)	0 (0%)	0.794
Recurrent stroke	0 (0%)	1 (4%)	0.505
Cerebral herniation	1 (3.7%)	1 (4%)	0.293
Gastrointestinal bleeding	1 (3.7%)	0 (0%)	0.466
Fever over 38^o c	0 (0%)	0 (0%)	1.00

In a cohort of 52 patients (25 receiving Fingolimod and 27 on standard therapy) distinct immunological patterns emerged. Among those treated with Fingolimod, CD4$^+$ T-cell counts declined from a median of 650 cells/μL (IQR 520–780) at baseline to 480 cells/μL (IQR 360–620) by day 3, representing a 26% reduction (p = 0.072). CD8$^+$ T-cells exhibited a more pronounced decrease, from 420 cells/μL

(IQR 350–510) to 290 cells/µL (IQR 220–370), corresponding to a 31% reduction ($p = 0.008$). Notably, this reduction differed significantly between T-cell subsets ($p = 0.04$). In contrast, patients in the standard therapy group maintained stable T-cell counts: $CD4^+$ cells changed marginally from 645 cells/µL (IQR 500–770) to 630 cells/µL (IQR 490–760) (2.3% change, $p = 0.62$), and $CD8^+$ cells from 415 cells/µL (IQR 340–505) to 405 cells/µL (IQR 330–495) (2.4% change, $p = 0.58$), with no significant differences between subsets ($p = 0.82$). Importantly, all T-cell values remained within established physiological ranges ($CD4^+$: 410–1590 cells/µL; $CD8^+$: 190–1140 cells/µL), and no clinical adverse effects were observed. The results are shown in figure 3.

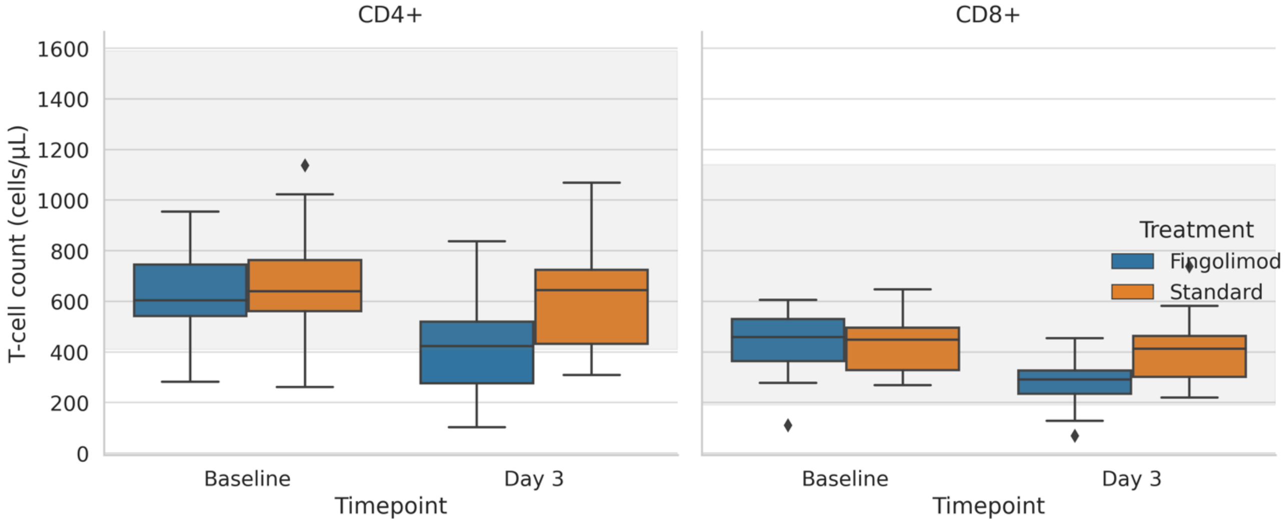

Figure 3 Leukocyte dynamics from baseline to day 3

Some patients with stroke had contraindications to the administration of Alteplase and received only Fingolimod. We compared the results between combination therapy (21 patients, 84%) and Fingolimod alone (4 patients, 16%) which were all previously analyzed as the Fingolimod group. A comparison of combination therapy and fingolimod alone showed no significant difference in the parameters of the study. No significant difference in post-stroke complications was seen between these subgroups. The details are demonstrated in Table 7.

Table 7 Comparison of the combination of Fingolimod and Alteplase versus Fingolimod alone

Parameter	Combination of Fingolimod and Alteplase group	Fingolimod group	P-value
Age	68.6±8.8	67.9±7.3	0.810
Gender			
- Male	13 (61.90%)	3 (75%)	0.409
- Female	8 (38.09%)	1 (25%)	
Underlying conditions			
Hypertension	11 (52.38%)	2 (50%)	0.311
Diabetes mellitus	6 (28.57%)	1 (25%)	0.457
Hyperlipidemia	9 (42.85%)	2 (50%)	0.302
Atrial fibrillation	2 (9.52%)	0 (0%)	0.781
Smoking	11 (52.38%)	2 (50%)	0.229
History of stroke	4 (19.04%)	1 (25%)	0.910
Medications			
Anti-platelets	4 (19.04%)	1 (25%)	0.610
Anti-coagulants	1 (4.76%)	0 (0%)	0.955
Onset-to-treatment time	3.1±0.2	3.0±0.3	0.880
Location of arterial occlusion			

Anterior cerebral artery	0 (0%)	0 (0%)	0.368
Proximal section of the first segment of middle cerebral artery	2 (9.52%)	0 (0%)	
The middle section of the first segment of the middle cerebral artery	1 (4.76%)	0 (0%)	
The distal section of the first segment of the middle cerebral artery	3 (14.28%)	1 (25%)	
The second segment of the middle cerebral artery	12 (57.14%)	3 (75%)	
Terminal internal carotid artery	3 (14.28%)	0 (0%)	
mRS, median (IQR)			
Baseline	4 (3-4)	4 (3-4)	0.877
30-day	2 (1-3)	2 (2-3)	0.461
90-day	2 (0-2)	2 (1-2)	0.569
NIHSS, median (IQR)			
Baseline	14 (7-25)	13 (9-19)	0.347
30-day	3 (0-16)	3 (1-5)	0.340
90-day	1 (0-12)	1 (0-3)	0.825

Discussion

In the present study, the efficacy of Fingolimod and Alteplase combination in treating acute ischemic stroke was evaluated. Our exploratory findings suggest that Fingolimod and Alteplase combination therapy may be associated with improved neurologic function parameters between 30 and 90 days after stroke compared to Alteplase alone. This combination was well-tolerated in our patients with no unexpected safety signals observed during the study period. However, given the small sample size and exploratory nature of this trial, these findings require confirmation in larger, adequately powered studies before definitive conclusions about efficacy and safety can be reported.

In our study, there was no difference between the two groups in terms of gender, age, underlying conditions, antiplatelets, anticoagulant consumption, onset-to-treatment duration, and location of arterial occlusion. Thus, these two groups are comparable and not confounded by these parameters.

mRS as a marker of neurologic function was not significantly different between the two groups at baseline but it was remarkably better at 30-day and 90-day periods in the Fingolimod group. mRS 0-2 at day 30 was 14.8% in the classic group and 52% in the Fingolimod group. These rates at day 90 were 22.22% and 66%, respectively. Tian et al (Tian et al., 2018) have shown that mRS 0-2 had increased from 22% at baseline to 57% at day 90 in the Fingolimod group. Zhu et al (Zhu et al., 2015) have shown better recovery at day 90 in the Fingolimod group compared to the Alteplase alone group. mRS 0-1 was 73% in the Fingolimod group and 32% in the classic group. Liantao et al (Liantao et al., 2019) have also reported that mRS scores were significantly lower in the Fingolimod group compared to controls.

NIHSS as a neurologic function factor was also not significantly different between the two groups at baseline. Thirty days after injection, there was a difference between the two groups, but this difference could not reach statistical significance (p=0.509). At day 90, the difference had become significant in favor of the Fingolimod group (p=0.026). Zhu et al. (Zhu et al., 2015) have reported significantly improved neurologic deficits in the Fingolimod group compared to controls (4 versus 2, p=0.04). Liantao et al. (Liantao et al., 2019) also reported significantly lower NIHSS scores in the Fingolimod group compared to controls at day 90 after stroke. Fu et al. (Fu et al., 2014) reported that one week after treatment, the reduction in NIHSS in the Fingolimod group was significantly more than in the classic treatment (4 vs. 1, p=0.001). As it can be concluded from our findings and other studies regarding mRS and NIHSS, Fingolimod has an evident impact on improving neurologic function after stroke. Two meta-analysis studies have confirmed this conclusion more comprehensively. They reported that neurobehavioral improvement has been 34.2% higher compared to controls. They also reported that out of 9 eligible studies, eight studies showed significant functional improvement with Fingolimod (Aljabali et al., 2023; Liu et al., 2013).

There was no difference between the two groups of our study in either early or late complications. The overall rate of complications was also negligible and comparable to other studies. Zhu et al (Zhu et al., 2015) and Fu et al (Fu et al., 2014) reported no serious adverse event related to the administration of Fingolimod combined with Alteplase. Two published meta-analyses also reported no significant difference between complications of Fingolimod and Alteplase. Thus, it can be concluded that Fingolimod is not significantly different from Alteplase regarding safety status.

The results of the effect size assessment demonstrate that Fingolimod treatment led to superior functional and neurological recovery compared to standard therapy, with effects becoming more pronounced over time. The robust effect sizes for mRS outcomes (rank-biserial r increasing from 0.33 to 0.38) coupled with the high odds ratios for achieving functional independence (ORs 6.21-7.44) suggest clinically meaningful benefits of fingolimod. Analyzing NIHSS, while neurological improvements were more modest, they followed a similar progressive pattern, reaching statistical significance by 90 days. The improving NNT values (from 3 to 2 for mRS and 5 to 4 for NIHSS) indicate increasing treatment efficiency with longer follow-up. This temporal pattern suggests that fingolimod may enhance both the speed and completeness of recovery. The consistent findings across different effect size measures (rank-based correlations and odds ratios) strengthen confidence in these results.

The fingolimod group demonstrated selective immunomodulation, with significant CD8+ T-cell reduction while maintaining relatively stable CD4+ counts, contrasting sharply with the stable lymphocyte profiles in the standard therapy group. This 31% CD8+ decrease (versus 2.4% in controls) suggests a specific mechanism of action, potentially related to fingolimod's known effects on lymphocyte trafficking. The preservation of CD4+ counts (26% decrease versus 2.3% in controls), combined with all values remaining within normal ranges, indicates a favorable safety profile. These differential effects on T-cell subsets may have therapeutic implications for conditions where selective immune modulation is desired, though further studies should explore the clinical relevance of these transient changes. The stability of both subsets in the standard therapy group confirms these findings represent true treatment effects rather than natural variation.

It should be mentioned that we tried to analyze per-protocol data, which also holds for the 4 patients who only received fingolimod without alteplase due to contraindications of administering Alteplase which showed no important findings, as it was predictable due to the very low number of data; so, it should be interpreted cautiously and should count as limitations of our study.

Our study had some limitations; one of these was the narrow time window of 4.5 hours after the onset of symptoms. Fingolimod has been used for longer periods after stroke. Thus, administration of Fingolimod in various post-stroke periods can yield different outcomes which can be evaluated and compared in the upcoming investigations.

Another important limitation of this study is its relatively small sample size and the associated low statistical power. This can be related to the possibility of failing to detect a true treatment effect where one exists. This is particularly relevant for outcomes such as CD4+ cell reduction (p = 0.072), which trended toward significance but did not reach the threshold, and may reflect insufficient power rather than a true absence of effect.

We did not assess infarct and brain lesion volumes on MRI in our study. These parameters have been considered important indices of response to treatment in previous studies. Future research should incorporate neuroimaging biomarkers, including lesion volume quantification and diffusion-weighted imaging, to provide objective measures of treatment response and correlate them with clinical outcomes.

Inflammatory cell count, their immigration to brain status after stroke, and the impact of Fingolimod on them are not evaluated in our study. Further studies should include comprehensive immunological profiling, including cerebrospinal fluid analysis and advanced flow cytometry, to elucidate the mechanistic pathways through which Fingolimod exerts its neuroprotective effects. Finally, immediate phase, 24-hour, and 1-week post-injection time points have shown interesting alterations in similar studies, but we did not assess our patients at these early follow-up intervals. Future investigations should incorporate more frequent early assessments to capture the dynamic immunological and clinical changes during the acute and subacute phases of stroke, which may provide critical insights into Fingolimod's therapeutic effects.

Conclusion

In this study, we evaluated the potential role of Fingolimod in combination with Alteplase for the treatment of acute ischemic stroke. Our exploratory findings suggest that this combination therapy may be associated with improved neurologic function parameters between 30 and 90 days post-stroke when compared to Alteplase alone. The combination therapy was well-tolerated and no unexpected safety signals were identified within the observed study period. Fingolimod monotherapy also showed similar trends compared to the combination therapy; however, given the very small number of patients in this subgroup, no definitive conclusions can be drawn. Importantly, due to the limited sample size and exploratory nature of this trial, these findings should be interpreted with caution. Further large-scale and multicenter studies are needed to confirm these results and to fully approve the therapeutic potential and safety profile of Fingolimod in acute ischemic stroke.

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